

RAPID EVOLUTION OF CUTICULAR HYDROCARBONS IN A SPECIES RADIATION OF ACOUSTICALLY DIVERSE HAWAIIAN CRICKETS (GRYLLIDAE: TRIGONIDIINAE: *LAUPALA*)

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Understanding the origin and maintenance of barriers to gene exchange is a central goal of speciation research. Hawaiian swordtail crickets (genus *Laupala*) represent one of the most rapidly speciating animal groups yet identified. Extensive acoustic diversity, strong premating isolation, and female preference for conspecific acoustic signals in laboratory phonotaxis trials have strongly supported divergence in mate recognition as the driving force behind the explosive speciation seen in this system. However, recent work has shown that female preference for conspecific male calling song does not extend to mate choice at close range among these crickets, leading to the hypothesis that additional sexual signals are involved in mate recognition and premating isolation. Here we examine patterns of variation in cuticular lipids among several species of *Laupala* from Maui and the Big Island of Hawaii. Results demonstrate (1) a rapid and dramatic evolution of cuticular lipid composition among species in this genus, (2) significant differences among males and females in cuticular lipid composition, and (3) a significant reduction in the complexity of cuticular lipid profiles in species from the Big Island of Hawaii as compared to two outgroup species from Maui. These results suggest that behavioral barriers to gene exchange in *Laupala* may be composed of multiple mate recognition signals, a pattern common in other cricket species.

KEY WORDS: Acoustic communication, biogeography, chemical communication, courtship, Hawaii, *Laupala*, premating isolation, sexual signals, speciation.

Identifying the courtship signals involved in mate choice is one of the central challenges to understanding behavioral evolution and, in many animals, speciation (Andersson 1994). Sexual communication is highly complex, often involving multiple mating cues and signal modalities (Johnstone 1996; Jennions and Petrie 1997; Kodric-Brown and Nicoletto 2001; Candolin 2003; Patricelli et al. 2003; Hebets and Papaj 2005). For example, Hawaiian *Drosophila*

utilize at least four different sensory modalities of communication during courtship—visual, vibrational (airborne and substrate), chemical, and tactile—all of which contribute to behavioral isolation among species in some way (Boake 2005). In addition, mating cues may be context dependent (e.g., Alonzo and Sinervo 2001; McLain 2005), and interactions among sexual signals may alter an individual's perception or preference for single cues (Endler 1992;

Hankison and Morris 2002; Wong et al. 2005; Bateson and Healy 2005). Furthermore, multiple cues can provide information about different aspects of mate quality (van Doorn and Weissing 2004). Therefore, an investigation of behavioral evolution among closely related species would benefit by considering multiple aspects of sexual communication.

Hawaiian swordtail crickets, genus *Laupala*, are among the most rapidly speciating animal groups yet identified (Mendelson and Shaw 2005). Speciation among these crickets has proceeded by the colonization of newly emerging volcanic islands and subsequent intra-island radiations (Otte 1994; Shaw 1996; Mendelson and Shaw 2005). Unlike examples of ecologically driven speciation (Funk 1998; Via 1999; Rundle et al. 2000; Nosil et al. 2002), the most closely related species of *Laupala* have diversified in secondary sexual traits but remain morphologically and ecologically similar. Therefore, the most closely related species have been hypothesized based on differences in acoustic signaling, in particular the pulse rate of male calling song (Otte 1994; Shaw 2000a), and genetic evidence supports these species delimitations (Shaw 1999, 2002; Parsons and Shaw 2001; Mendelson and Shaw 2002, 2005). Multiple species often exist sympatrically, and calling (mating) times frequently overlap (Otte 1994; Shaw 1995); however, strong prezygotic barriers isolate most species of *Laupala*. Females are preferentially attracted to conspecific pulse rates (Shaw 2000b; Shaw and Herlihy 2000; Mendelson and Shaw 2002), and males of sympatric species always call with distinct pulse rates (Shaw and Danley 2003; Shaw, unpublished). These data collectively suggest that divergence among species of *Laupala* is driven largely by evolution of male calling song, and that sexual selection on this trait may be responsible for the rapid speciation rates observed among these crickets.

Recent data, however, suggest a composite architecture to sexual communication in this genus. Courtship in *Laupala* takes place over the course of several hours and involves a complex series of behavioral elements that are repeated multiple times (Shaw and Lugo 2001). Each courtship unit culminates in the passage of a spermless “microspermatophore” to the female and the mating terminates only after the transfer of a much larger “macrospermatophore,” which contains all of the sperm for a given mating (Shaw and Khine 2004; de Carvalho and Shaw 2005). Throughout this close-range courtship, males sing with the same song used for long-range calling. (Unlike many cricket species, *Laupala* males produce only a single, stereotypical song that is used for all social interactions [Shaw, unpublished]). Although female *Laupala* show a clear acoustic preference for conspecific pulse rates in phonotaxis trials (Shaw and Herlihy 2000; Shaw 2000b; Mendelson and Shaw 2002), a recent study revealed that this preference has no detectable effect on mate choice at close range (Mendelson and Shaw 2006). Females of two species that exhibit strong be-

havioral isolation showed no preference for hybrid males with pulse rates more similar to those of conspecific males. Rather, females in this study accepted hybrid males with pulse rates well outside the natural range of conspecifics (Mendelson and Shaw 2006).

The lack of correspondence between female preferences in phonotaxis trials and for singing males at close range suggests that *Laupala*, like other crickets (Balakrishnan and Pollack 1997), rely upon additional sexual recognition cues during close-range courtship. Behavioral observations also support this hypothesis. For instance, male singing during courtship is always preceded by initial antennal contact, and production of each spermatophore occurs while males and females “face” each other and engage in extensive antennal interactions (Shaw and Lugo 2001; Shaw and Khine 2004). Thus chemical and/or tactile cues may be exchanged during courtship, and behavioral isolation may be a function of multiple mating signals, rather than simply a by-product of long-range attraction of females to conspecific male songs.

Chemical communication among insects is extremely widespread, particularly among social insects (see Vander Meer et al. 1998) and sexual pheromones are well known from many insect groups (e.g., *Drosophila*, Ferveur 2005; *Blattella*, Nojima et al. 2005; Pherobase: <http://www.pherobase.com/>; Pherolist: <http://www.pherolist.slu.se/pherolist.php>). Cuticular hydrocarbons (CHCs), in particular, serve as mate recognition signals in many insects (reviewed by Howard and Blomquist 2005). Although the primary function of CHCs is to prevent desiccation, in the Orthoptera these chemicals have been shown to function in kin recognition (Simmons 1990), mate recognition (Tregenza and Wedell 1997), and as indicators of both immunocompetence (Rantala et al. 2002, 2003) and dominance (Rantala et al. 2004; Kortet and Hedrick 2005). They have been particularly well-studied in locusts, where they are known to influence a range of social behaviors including social cohesion, gregariousness, mating, oviposition, and maternal transfer of gregarious characters (reviewed in Hassanali et al. 2005). In addition, variation in CHCs across a hybrid zone between subspecies of *Chorthippus* meadow grasshoppers in Europe (Neems and Butlin 1994, 1995; Buckley et al. 2003) is associated with levels of assortative mating in this species (Tregenza et al. 2000), suggesting that reproductive barriers may arise as a by-product of evolution in these traits.

Therefore, it is possible that cuticular chemicals represent a previously unrecognized mode of sexual communication among Hawaiian *Laupala*. To investigate this possibility, we assayed and statistically analyzed chemical variation in cuticular lipids among natural populations of several species of the “*pacifica*” species group from the Big Island of Hawaii and Maui, including the most rapidly diverging clade of the genus *Laupala* (Mendelson and Shaw 2005).

Materials and Methods

SAMPLING AND SPECIES IDENTIFICATION

Approximately 5–10 individuals ($n = 71$, 50 males and 21 females) of eight different *Laupala* species were sampled from the Big Island of Hawaii (6) and Maui (2) during the summer of 2005. Sampled populations included Double Helix (*L. hualalai* and *L. kona*), Manuka (*L. kona*), Kaiwiki (*L. parangira*), Eucalyptus Toe (*L. nigra*), Kupehau II (*L. kohalensis*), Naulu and Glenwood Road (*L. pruna*) and, on Maui, Palikea North and Gingercamp (*L. makaio*) and Kipahula (*L. orientalis*). Specimens were hand collected in the field by net and transferred to glass containers with aluminum foil tops to prevent contamination of cuticular lipids with plastic residues. Because species of *Laupala* are ecologically and morphologically cryptic, collecting was done from previously sampled localities with known species composition. Recording and analyzing male songs prior to use for chemical analysis further confirmed species identity (song analysis followed Shaw 1996). In addition, we crossed lab-reared individuals of two species (*L. paranigra* male \times *L. kohalensis* female) to generate F1 and F2 hybrids, and used these progeny to determine whether variation in cuticular composition was primarily influenced by genetic versus environmental factors.

EXTRACTION OF CUTICULAR LIPIDS

Crickets were first anesthetized with CO₂ and the lipids were extracted using HPLC-grade hexane. All glassware was pre-rinsed three times with hexane to remove possible contaminants and allowed to dry in a fume hood for 5 to 10 min prior to beginning extractions. Between each extraction, forceps were rinsed in three separate containers of wash hexane to avoid cross-contamination of samples. Each cricket was submerged in 1 ml of hexane for 5 min in pre-washed glass vials with Teflon-lined caps. At the end of 5 min, each vial was swirled and the cricket was removed with forceps into 1.5 ml Eppendorf tubes for later DNA extraction.

GAS CHROMATOGRAPHY

Dried extracts were reconstituted in *n*-hexane for chemical analysis and 1.5- μ l aliquots were assayed via gas chromatography (Hewlett Packard 5890, equipped with flame ionization detector). Separation of the volatilized components was performed on a fused silica capillary column (DB-5: 30 m \times 0.25 mm \times 0.5 μ m film thickness). Ultra high purity helium was used as carrier gas at 150 kPa, corresponding to an average linear velocity of 30 cm sec⁻¹. The hexane extracts were injected into a splitless injector (300°C) at an initial oven temperature of 100°C (held for 2 min). The oven temperature was then ramped to 310°C at 10°C min⁻¹ and held for 20 min, resulting in a total run time of 43 min. The flame ionization detector was held at 310°C. Chromatograms

were acquired and analyzed using ChemStation software (Agilent, Foster City, CA).

STATISTICAL ANALYSIS OF GAS CHROMATOGRAPHY RESULTS

We identified and analyzed a total of 34 different gas chromatographic peaks, each of which represented more than 1% of the total area in all individuals of at least one species of *Laupala* sampled. Chromatogram peaks were scored conservatively based on their retention index; therefore, in some cases a scored peak may represent more than one hydrocarbon. Peak areas were determined as a function of the total area counts for all 34 peaks for a given individual and were standardized to 100%. The compositional percent peak areas were transformed (see Aitchinson 1986) using Reyment's (1989) formula, $Z_{i,j} = \log(X_{i,j}/g(X_j))$, and subjected to multivariate analysis following Liebig et al. (2000): $X_{i,j}$ = the area of peak i for individual j , $g(X_j)$ = the geometric mean of area of all peaks for individual j , and $Z_{i,j}$ = the transformed area of peak i for individual j .

A principal components analysis, performed in JMP 6.0 (SAS Institute, Cary), was used to reduce the dimensionality of the describing variables (CV1-8: eigenvalues >1). The extracted factors that best explained the variation in the data were then used in a discriminant function analysis (DFA) to separate species using data from both males and females. A similar analysis was also performed to assess differences between male and female *L. makaio*; this species was chosen because previous work (Shaw and Lugo 2001) has shown that asymmetrical sexual isolation occurs between Maui endemic *L. makaio* and a derived species, *L. paranigra*, from the Big Island.

Results

One of the primary goals of this study was to test the hypothesis that variation exists among *Laupala* species in the composition of their cuticular lipids, as expected if these chemicals are involved in sexual communication. Consistent with our expectations, we found dramatic qualitative and quantitative differences in the chemical profiles of all the species we sampled (Fig. 1). Although each species of *Laupala* on the Big Island has a relatively small number of gas chromatography peaks (~7) compared with other crickets (15 or more; Tregenza and Weddell 1997), variation in the presence and absence of different peaks allows species to be distinguished easily.

DISCRIMINANT ANALYSIS OF LAUPALA SPECIES

Principal components analysis reduced the dimensionality of the peak area data to eight canonical variates that explained 97.1% of

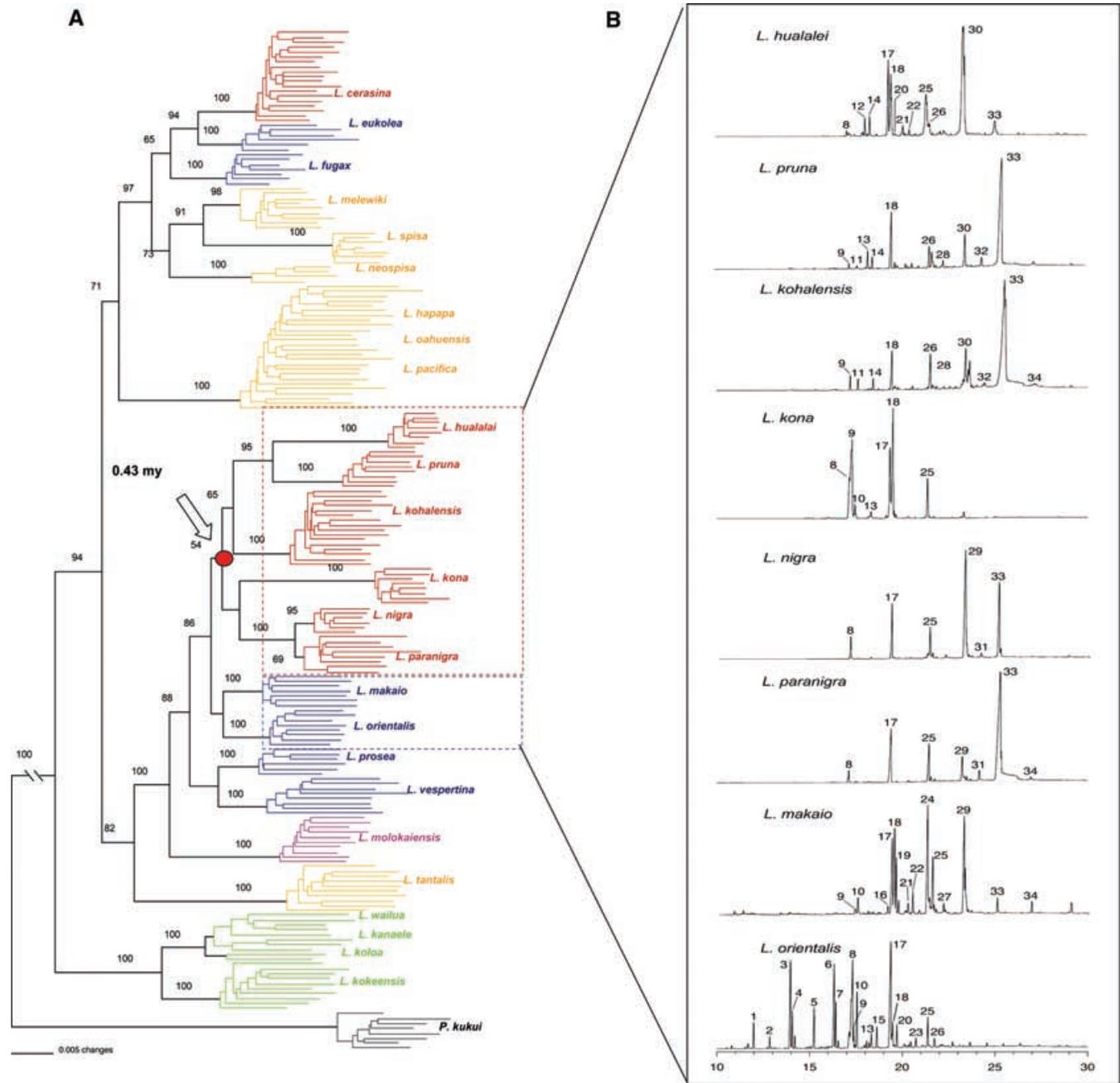


Figure 1. (A) Fully resolved AFLP phylogeny showing the geographic distributions of each species. Terminal taxa are individuals; branches are color-coded to indicate Hawaiian island of origin (green, Kauai; yellow, Oahu; purple, Molokai; dark blue, Maui; red, Hawaii – Mendelson and Shaw 2005), (B) Representative GC profiles of cuticular compounds for each of the “*pacifica*” group species on the Big Island. Numbers represent individual characters, but a peak was only scored for an individual if it represented greater than 1% of the total area for all peaks. Note both the striking differences among these closely related species and the strong similarity among sister taxa.

the variation among these species; additional factors did not improve the discriminant function and had eigenvalues below 0.25. Using the first two of these extracted factors (CV1 and CV2), respectively, 37.0% and 25.9% of the variation, a DFA was able to correctly assign all 71 individuals to the species level; species designations had been previously determined using a combina-

tion of male calling song, collecting locality, and prior phylogenetic sampling. Most of the separation is due to CV1, but *L. orientalis* and *L. makaio* fall at opposite extremes along CV2 (Fig. 2a). The MANOVA results indicate statistically significant differences (Wilks' $\lambda = 0.0000336$, $F = 1286.5$, NumDF = 16, DenDF = 120, $p < 0.0001$) among *Laupala* species in their

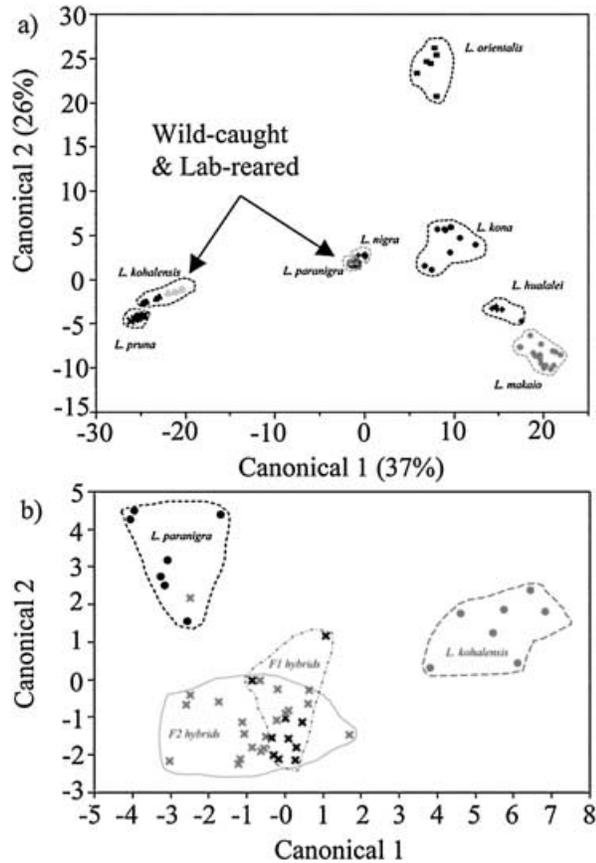


Figure 2. (a) DFA of wild-caught individuals ($n = 71$) based on two canonical variates; lab-reared representatives of *L. parangira* ($n = 7$) and *L. kohalensis* ($n = 7$) included for comparison. Significant differences exist among each species' cuticular lipids ($P < 0.0001$). Dashed lines represent 95% confidence limit for the group mean, calculated in JMP 6.0. (b) DFA for lab-reared *L. parangira* and *L. kohalensis* ($P < 0.0001$). Note that two hybrid groups are intermediate relative to parentals and overlap extensively.

chemical profiles with the exception of the closely related species, *L. parangira* and *L. nigra*. However, if an additional factor is used (CV5 or CV6), it becomes possible to separate these sister species. Although the species-level analysis combined data from both males and females, additional analyses using only males or only females produced similar results.

STATISTICAL COMPARISON OF WILD-CAUGHT AND LABORATORY-REARED INDIVIDUALS

A secondary analysis of laboratory-reared *L. parangira* ($n = 7$) and *L. kohalensis* ($n = 7$) found that they share all of the major peaks of conspecific wild-caught individuals and group together with wild-caught conspecifics when included in the species-level DFA using the first two canonical variates (Fig. 2a). No significant differences were found between lab-reared and wild-caught individuals of *L. parangira*. In contrast, significant differences

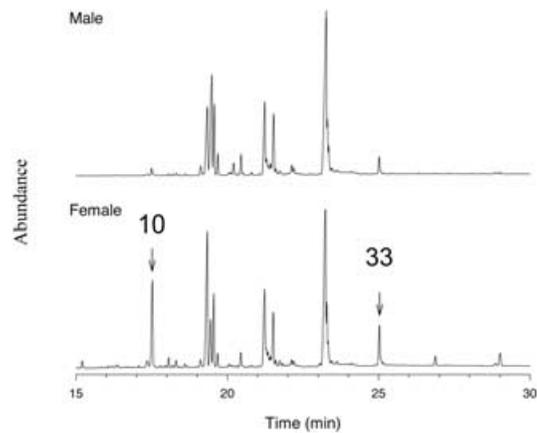


Figure 3. Gas chromatograms for male and female *L. makaio* samples (numbered arrows indicate peaks that differ significantly after correction for multiple tests).

separate lab-reared versus wild-caught specimens of *L. kohalensis*, but these two populations cluster tightly with each other in both the DFA (Fig. 2a) and in distance-based hierarchical clustering analyses (not shown).

To further assess whether a genetic basis underlies the observed variation in cuticular lipids, we also conducted a statistical comparison (DFA) of lab-reared *L. parangira*, *L. kohalensis*, and their F1 (*L. parangira* male \times *L. kohalensis* female; $n = 10$) and F2 (male F1 \times female F1; $n = 21$) hybrid progeny. The results of this analysis demonstrate that hybrids have intermediate GC profiles relative to lab-reared parentals and, as expected, F2 offspring display a wider range of variation than F1 individuals (Fig. 2b).

CHEMICAL DIFFERENCES BETWEEN MALES AND FEMALES

We also tested whether significant differences in cuticular composition exist between males and females of *L. makaio*, the species for which we had the largest sample size ($n = 17$, 10 males, 7 females). We grouped the sampled individuals by sex and, as above, utilized a discriminant analysis based on the extracted principle component factors. Statistically significant differences ($p < 0.001$) were found between males and females using a standard DFA with canonical variates CV1–CV3 ($\sim 75\%$ of the total variation). Pairwise one-way ANOVA results indicate that GC peaks 10 (DF = 1, F -ratio = 10.4890, $P = 0.005$, $\alpha = 0.005$) and 33 (DF = 1, F -ratio = 19.7323, $P = 0.0009$, $\alpha = 0.0015$) significantly vary between males and females of this species after sequential Bonferroni correction for multiple tests (Fig. 3).

CHEMICAL DIVERSITY AMONG SPECIES

Direct comparison of cuticular profiles among species (Fig. 1) demonstrates that individuals sampled from the ancestral Maui species (*L. makaio* and *L. orientalis*) possess more than twice as

many major GC peaks on average than species sampled from the more recently derived lineage on the Big Island of Hawaii (Maui $\bar{\chi} = 15$, S.D. = 1 versus Big Island $\bar{\chi} = 7$, S.D. = 2.13; Fig. 1). Examination of the gas chromatograms derived from the Big Island species relative to their known phylogenetic relationships (Fig. 1) highlights the strong similarity among closely related taxa with respect to their cuticular profiles while also emphasizing the clear qualitative differences in the composition of each species cuticular lipids.

Discussion

Courtship phenotypes are among the first traits to evolve among incipient species (Gleason and Ritchie 1998). In particular, CHCs have been shown to differ very early in lineage divergence and are nearly ubiquitous in insects (Howard and Blomquist 2005). The evolution of CHCs as mating signals is therefore likely to be extremely important in insect speciation.

SPECIES-SPECIFIC DIFFERENCES

Our results demonstrate that species of Hawaiian *Laupala* display considerable diversity in the composition of their cuticular lipids. We found significant differences among all sampled species, and both the discriminant and cluster analyses indicate that these differences extend to the population level in at least some cases. These results are remarkable for two reasons. First, species of *Laupala* are ecologically and morphologically cryptic, and all species are dietary generalists (Otte 1994). Although floral compositions of the habitat may differ, all species exist in mid-elevation rain forests, and appear to survive equally well in both native and introduced forests. Indeed, until now, the only significant phenotypes known to vary among the most closely related species were traits of the acoustic system. This study, therefore, reveals a cryptic but equally distinct phenotype that may also be involved in sexual communication. Second, speciation among the Big Island species of *Laupala* is both explosive and ongoing (Mendelson and Shaw 2005). The rapid divergence of the Big Island “*pacifica*” group has occurred during the past 430,000 years, at most, suggesting extremely rapid evolution of cuticular hydrocarbon diversity.

Our results reveal that CHC groupings correspond with pulse rate, genealogical distinctiveness, and previously delineated species boundaries (Otte 1994; Shaw 2000a, 2002; Mendelson and Shaw 2005). Hypothesizing a genetic basis for variation in CHC composition, we may conclude that closely related individuals have similar CHC profiles due to shared genetic history. Work on *Drosophila* (Coyne et al. 1994; Takahashi et al. 2001; Gleason et al. 2005) has shown that variation in cuticular compounds is the result of polygenic inheritance, and the extensive use of CHCs for taxonomic purposes (e.g., Page et al. 1997; Buckley et al. 2003) assumes a genetic basis to variation in CHCs. However, CHCs are

also heavily influenced by environmental conditions such as humidity and temperature (Noorman and Den Otter 2002), rearing environment (Stennett and Etges 1997; Etges and Ahrens 2001), and diet (Liang and Silverman 2000). Thus, a second possibility is that because we sampled species at a single or few locations, chemical variation simply corresponds to environmental, rather than genetic, variation.

Three lines of evidence allow us to reject an environmental explanation for the patterns observed. First, the DFA identified sympatric populations of *L. hualalai* and *L. kona* as distinct clusters (Fig. 2a). Thus individual crickets from the same location have chemical profiles more similar to conspecifics than to sympatric heterospecifics. To the extent that *L. hualalai* and *L. kona* share ecological conditions, these results support a genetic basis to their CHC differences. Second, lab-reared individuals cluster with wild-caught individuals in all analyses (Fig. 2a). This finding strongly supports the view that genetic factors have a greater influence than environment on phenotypic variation among species. Finally, hybrids show intermediacy along the first canonical axis relative to parentals (Fig. 2b), a pattern expected for quantitative traits. Thus we can conclude that there is a significant genetic contribution to the phenotypic variation among species in CHCs, although environmental factors may also contribute to this variation.

SEXUAL DIFFERENCES IN CUTICULAR LIPIDS

In addition to dramatic variation among species, we also found significant differences in the cuticular chemistry between the sexes (Fig. 3), consistent with the hypothesis that CHCs function as a mate recognition signal in *Laupala*. Similar quantitative differences between male and female field crickets (*Gryllus bimaculatus*) have been shown to mediate mate choice (Tregenza and Wedell 1997), assessments of male immunocompetence quality (Rantala et al. 2002, 2003), and dominance (Kortet and Hedrick 2005). Behavioral observations provide ancillary evidence that *Laupala* are using chemical cues for mate recognition. During mating trials, antennal contact stimulates initiation of male singing behavior and courtship often proceeds rapidly after first contact (Shaw, unpublished). Thus, chemical cues are likely to play an important role in sexual communication in this system and may provide an explanation of Mendelson and Shaw’s (2006) result that female pulse-rate preferences at long range do not extend to close-range mate choice of hybrid males. However, additional work needs to be done to demonstrate that *Laupala* are using a combination of acoustic and chemical sexual signals as mate recognition cues.

IMPLICATIONS FOR BEHAVIORAL EVOLUTION

Examining the distribution of CHC variation across a recently published molecular phylogeny for *Laupala* (Fig. 1) reveals that

all closely related Big Island species are similar in having relatively few major chemical peaks, whereas both Maui species are characterized by a relatively greater number of peaks. For example, *L. makaio* from the geologically older island of Maui has over twice as many chemical peaks as *L. paranigra* from the geologically younger Big Island of Hawaii. One interpretation of these findings is that there has been a simplification in the chemical composition since the founding and radiation of Big Island species due to drift and/or founder effects. If CHCs function in mate recognition, however, such changes could represent a founder-induced loss of courtship elements that may influence the direction of premating compatibility, as hypothesized by Kaneshiro (1976, 1980); crosses between *L. makaio* and *L. paranigra* show sexual isolation asymmetry in the direction predicted by the Kaneshiro hypothesis (Shaw and Lugo 2001). More extensive sampling for outgroup comparisons will be required to rule out the alternative possibility that the complexity in the Maui taxa is a derived condition. In addition, ongoing research on the chemical identity of each peak will help illuminate patterns and processes of CHC evolution. However, given what is known from other insects (Howard and Blomquist 2005), the difference in complexity of CHCs is likely due to a reduction in the complexity of modifications of straight-chain hydrocarbon, rather than an absence of particular hydrocarbon groups (see also Ferveur 2005).

Divergent natural selection related to environmental conditions remains an additional explanation as the driving force in the evolution of CHCs in this system (Tregenza et al. 2000). Although no obvious ecological or morphological differences separate cricket species, local environmental variation may influence CHC evolution among populations. Thus, a combination of natural and sexual selection could be involved in CHC evolution. If, like many other insects, *Laupala* use variation in CHCs as mate recognition cues, then adaptation to local environmental conditions may incidentally result in divergence in mating preferences among populations. Subsequently, sexual selection could act to amplify this initial premating isolation among locally adapted populations. Theoretical work (Lande 1982; Endler 1992; Schluter and Price 1993) and, more recently, experimental studies (Boughman 2001; Kwiatkowski and Sullivan 2002; Nosil et al. 2002) have shown that sexual selection can drive the evolution of premating isolation following initial divergence through natural selection between two populations. For example, Rundle et al. (2005) recently demonstrated that female mating preferences in lab-reared populations of *Drosophila serrata* evolve as a by-product of divergent environmental selection on CHC profiles due to local adaptation to larval diet. Although it remains to be shown that *Laupala* rely on chemical cues for mate recognition, it is possible that natural selection could drive CHC divergence in this group and thereby contribute to its rapid speciation rate.

CONCLUSIONS

Although the functional roles are not well understood, the use of multiple mating cues or signaling modalities in sexual communication is common (Johnstone 1996; Jennions and Petrie 1997; Kondric-Brown and Nicoletto 2001; Candolin 2003; Patricellie et al. 2003; Takahashi and Ting 2004). Our results demonstrate that cuticular lipids represent a previously unrecognized phenotype that is evolving as rapidly as male-calling song among morphologically and ecologically cryptic species of Hawaiian *Laupala* crickets. Given the recent finding that female preferences for male calling song do not extend to mate choice (Mendelson and Shaw 2006) in this system, and the fact that courtship is highly elaborate, our results suggest that behavioral barriers to gene exchange in *Laupala* may be composed of multiple mate recognition signals representing at least two sensory modalities; a pattern common in other cricket species (e.g., Balakrishnan and Pollack 1997; Tregenza and Wedell 1997). If so, then it is possible that these signals act at different stages of *Laupala* courtship and may combine to increase the total isolation among lineages. Female acoustic preference may operate early in courtship as a long-range behavioral reproductive barrier, whereas short-range sexual communication may involve a bouquet of chemical and tactile mating cues that together act to isolate different species of these crickets. Future work will focus on male courtship behavior in response to female cuticular hydrocarbon profiles, female preference for male chemical cues, and a quantitative genetic study of variation underlying this trait.

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